U.S. PATENT APPLICATION

Inventor(s):   Alan D. Schreiber

Invention: METHOD OF TREATING ATHEROSCLEROSIS

NIXON & VANDERHYE P.C.
ATTORNEYS AT LAW
1100 NORTH GLEBE ROAD
8TH FLOOR
ARLINGTON, VIRGINIA 22201-4714
(703) 816-4000
Facsimile (703) 816-4100

SPECIFICATION

METHOD OF TREATING ATHEROSCLEROSIS

This application claims priority from Prov. App. No. 60/126,407, filed March 26, 1999. The entire contents of the provisional application is

5 incorporated herein by reference.

TECHNICAL FIELD

The present invention relates to a method of treating atherosclerosis, and to compounds and compositions suitable for use in such a method.

10 ## BACKGROUND

Delayed development of coronary artery disease in premenopausal women, compared to that of age-matched men, was noted over sixty years ago (Levy et al, J. Am. MedAssoc. 107:97-102 (1936), Master et al, Arch.

15 Int. Med. 64:767-786 (1939)). Despite numerous reports addressing the antiatherogenic effect of estrogen in premenopausal women and in postmenopausal women on estrogen replacement therapy, the mechanism of this protective effect and its relationship to

20 alteration in serum lipids is still unclear.
Even less is known about the effect of progesterone on the development of atherosclerosis, either alone or in combination with estrogen. Conflicting clinical and experimental data suggest

25 that the effect of exogenous progestogens on the

development of coronary artery disease ranges from
adverse (Hirvonen et al, N. Engl. J. Med. 304:560-563
(1981)) to null (Adams et al, Arteriosclerosis
10:19051 (19090)) to potentially desirable (Haarbo et

5   al, Am. J. Med. 90:584-589 (1991)) to favorable
(Alexandersen et al, Arterioscler Thromb. Vasc. Biol.
18:902-907 (1998)) when combined with estriol
treatment. A favorable effect of progesterone
treatment alone is not believed to have been

10 documented in a clinical study.

Various animal models used to study the effects
of sex steroids on the development of atherosclerosis
have demonstrated beneficial results associated with
exogenous estrogen with or without progesterone in

15 chickens (Pick et al, Circulation 6:276-280 (1952),
Pick et al, Circulation 4:468 (1951)), mice (Sullivan
et al, J. Clin. Invest. 96:2482-2488 (1995)), rabbits
(Kushwaha et al, Metabolism 30:359-366 (1981), Fischer
et al, Atherosclerosis 54:177-185 (1985), Haarbo et

20 al, J. Clin. Invest. 87:1274-1279 (1991), Hanke et al,
Circulation 94:175-181 (1996), Holm et al, Circulation
98:2731-2737 (1998)), monkeys (Wagner et al, J. Clin.
Invest. 88:1995-2002 (1991)), and baboons (Kushwaha et
al, Arterioscler. Thromb. 11:23-31 (1991)). Spagnoli

25 and coworkers (Spagnoli et al, Atherosclerosis 82:27-
36 (1990)) reported for the first time that high-dose
synthetic progestogens alone inhibited atherosclerotic
plaque formation in female rabbits fed a cholesterol-

- 2 -

enriched diet and concluded that the protective effect
was only partly due to alteration in serum
cholesterol.

The present invention results, at least in part,
5   from the observation that aortic atherosclerotic
plaque load can be decreased in hypercholesterolemic
male rabbits with exogenous progesterone as well as
with estriol, the decreases in plaque load being
independent of alterations in serum lipid levels.

10   SUMMARY OF THE INVENTION

The present invention relates to a method of
treating atherosclerosis. The method comprises
administering to a patient in need thereof an
effective amount of a progestational agent that has a
15   minimal effect on sex organs or a non-steroidal
compound that inhibits macrophages or macrophage
function.

Objects and advantages of the present invention
will be clear from the description that follows.

20   BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the stucture of haloperidol, SCH-
23390 and sulpiride.

- 3 -

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of treating atherosclerosis (including atherosclerotic cardiovascular disease and stroke) in a mammal (e.g., a human). The method comprises administering to a mammal suffering from atherosclerosis an effective amount of a progestational agent that has a minimal effect on sex organs (e.g., less of an effect that medroxyprogesterone acetate) or a non-steroidal compound that inhibits macrophages or macrophage function (e.g., that inhibits macrophage Fc receptors).

Progestational agents suitable for use in the invention include the progesterone analogs of USP 4,902,681 and 4,908,358. A preferred such agent is 17-hydroxyprogesterone.

Non-steroidal compounds suitable for use in the present invention include benzazepines and butyrophenones, and pharmaceutically acceptable salts thereof. Benzothiophenes described in USP 5,075,321 can also be used. SCH-23390 is an example of a suitable benzazepine and haloperidol is an example of a suitable butyrophenone (see Figure 1). Analogues of, for example, SCH-23390 or haloperidol, that is, compounds having a similar structure or similar binding affinity for dopamine receptors or comparable capacity to antagonize or stimulate dopamine

- 4 -

receptors, can also be used. Structurally similar
structures include those wherein an alternative
halogen is present as a substituent (e.g., F rather
than Cl in the case of SCH-23390) or an alternative C_1-

5   C_4 alkyl substituent is present (e.g., $-CH_2CH_3$ rather
than $-CH_3$ in the case of SCH-23390). Benzazepines and
butyrophenones have been shown to bind dopamine
receptors (SCH-23390 is a D_1 antagonist while
haloperidol is a D_2 antagonist (Sunahara et al, Br. J.

10   Psych. 163(suppl. 22):31 (1993)). Other D_1 and D_2
antagonists can be used in accordance with the present
method.

     Compounds of the invention can be formulated into
pharmaceutical compositions suitable for

15   administration via a variety of routes. For example,
the compositions can be suitable for oral, rectal,
intravenous, or parenteral administration. The
compounds can also be formulated so as to be suitable
for administration via inhalation. Compositions

20   suitable for such forms of administration are,
advantageously, sterile.

     Depending on the intended mode of administration,
the pharmaceutical composition can be in the form of
solid, semi-solid or liquid dosage form, such as, for

25   example, a tablet, pill, capsule, powder, liquid,
suspension, or the like, preferably in unit dosage
form suitable for single administration of a precise
dosage. The pharmaceutical composition can include a

- 5 -

conventional pharmaceutical carrier or excipient and a compound according to the invention as an active ingredient. In addition, it can include other medicinal or pharmaceutical agents, carriers,

5   adjuvants, etc.

For oral administration, which is preferred, the pharmaceutical composition can take the form of a solution, suspension, tablet, pill, capsule, powder, sustained release formulation or the like.

10   For solid pharmaceutical compositions, conventional non-toxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and

15   the like. Liquid pharmaceutically administrable compositions can be prepared by dissolving or dispersing, or otherwise preparing a compound according to this invention and mixing it optionally with a pharmaceutical adjuvant in a carrier, such as,

20   for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension.

Methods of preparing compositions with a certain amount of active ingredient are known, or will be

25   apparent, to those skilled in this art. For example, see Remington's Pharmaceutical Sciences., Mack Pub. Co., Easter, Pa., 15th Ed. (1975).

Dosage regimens suitable for use in the present

- 6 -

330629

invention can be selected so as to achieve the clinical response (e.g., reduction in atherosclerosis plaque load) sought. The doses used will vary depending, for example, on the specific compound employed, the status of the patient and the route of administration. While optimum doses can be readily determined, dosages will generally be selected such that local blood concentrations of 100 to 200,000 pg/ml are achieved, 1,000 to 100,000 pg/ml being preferred, and 10,000 to 100,000 pg/ml being most preferred.

The following non-limiting Examples describes certain aspects of the invention in greater detail.

EXAMPLE 1

EXPERIMENTAL DETAILS

Male New Zealand White rabbits (3.2-4.9 pounds each) were fed a 0.5% cholesterol chow diet for 12 weeks and randomly assigned to five groups (n=6 each): one control group (CG) and four groups treated with estriol (E), haloperidol (H), low-dose 17-hydroxyprogesterone (LDP), or high-dose 17-hydroxyprogesterone (HDP). Serum cholesterol (C) and triglyceride (T) were measured before and after the treatment period in all groups; serum progesterone was determined only in the LDP and HDP groups. After the treatment period, rabbits were euthanized for

- 7 -

histomorphometric analysis of aortic plaque load. The
rabbits were treated according to the Animal Welfare
Act specifications.

Drug Administration

5   Vehicle only (CG) and vehicle plus steroid
(treated groups) were administered daily
subcutaneously. Steroid doses were as follows:
estriol, 1mg/kg; haloperidol, 1mg/kg for 2 weeks,
10 mg/kg for 2 weeks, and 5 mg/kg for 8 weeks; low-

10   dose 17-hydroxyprogesterone, 10 mg/kg; high-dose
17-hydroxyprogesterone, 90 mg/kg. The dose of
haloperidol was reduced from 10 mg/kg because of the
rabbits' poor tolerance of the higher dose.

Histomorphometric Analysis

15   Five-micron, serial histologic sections (15 each)
of 27 ascending aortas were cut from paraffin blocks,
stained with hematoxylin and eosin (H&E) and were
studied by light microscopy. Images of tissue
sections were captured to a Power Macintosh 7300/200

20   computer by a Hitachi 3-CCD Color Camera (model
HV-C20) attached to a Nikon Eclipse E600 microscope.
With digital imaging (IPLap Spectrum, Signal Analytics
Corporation, Vienna, VA), they were then analyzed by
manual color segmentation by tracting the endothelial

25   surface (intima), internal elastic lamina, and
external elastic lamina of each vessel. From

- 8 -

segmented images, intimal and medial areas were computed, allowing calculation of intima to media ratios. Plaque load for each aorta was defined as the ratio of intimal area to medial area (I/M).

5   Statistical Analysis

Serum lipid levels and I/M were expressed as the mean+SEM for all animals. The changes in lipid levels before and after the treatment period were compared using paired t-tests. The differences between the

10   treatment groups and controls were compared using analysis of variance (ANOVA) techniques. Repeated Measures ANOVA was used to compare the difference in plaque loads among the five groups. Multiple regression models were used to compare the differences

15   in plaque loads between the four treatment groups and the control group, adjusting for the difference in cholesterol and triglyceride levels. $P<0.05$ was considered statistically significant.

RESULTS

20   Alteration of Serum Lipids

Over the treatment period, mean serum C increased significantly in all groups. In the HDP group, the increase in serum C (cholesterol) was even greater than that of the CG. Serum T (triglyceride) decreased

25   slightly in the E group but significantly increased in the other groups. Post-treatment serum P levels were

- 9 -

significantly higher than baseline levels in the LDP
and HDP groups.   These data are summarized in Table 1.

Gross and Microscopic Findings

After 91-100 days of treatment, twenty-seven
5   rabbits were euthanized for histomorphometric analysis
of aortic plaque load.   One rabbit in the E group died
after 18 days of treatment, and two rabbits in he
H group were sacrificed at 39 and 71 days because of
their failure to thrive.

10   Gross examination of the aortas revealed
decreased atherosclerosis in the progesterone and
estrogen treated animals.   With the control animals,
there was about 42% occlusion.   With the low dose
progesterone there was 6% occlusion and with the high
15   dose progesterone there was 0% occlusion evident.

Fifteen stained serial histologic sections of
ascending aorta from each of the twenty-seven
surviving rabbits were examined by light microscopy.
Atherosclerotic plaques in all specimens revealed
20   variably dense aggregates of macrophage foam cells and
α-actin positive smooth muscle cells in a thickened
intimal matrix.   Rounded foam cells were commonly seen
straddling both sides of the focally degenerated
internal elastic lamina.

Analysis of Aortic Plaque Loads

Study of the mean I/M for each group, determined by computerized image analysis, revealed significant decreases in all four treatment groups (P<0.001 for E, LDP and HDP, and P=0.02 for H) when compared to the CG, as shown in Table 2. As further shown in Table 3, both LDP and HDP decreased the atherosclerotic plaque.

Even though the change in serum C and T levels did not have strong relationships with I/M, the values were quite different among the five groups. The group differences were examined, adjusting for the changes in serum C and T levels using a multiple regression model. The aortic plaque load remained lower in all four groups that that of the controls, E (P=0.052), H (P=0.069), LDP (P=0.026), HDP (P=0.014), after controlling for the changes in serum C and T levels; the plaque load difference approached statistical significance in the E group and reached the significance level in the HDP group.

330629

Table 1. Quantitative Serum Lipid Analysis

		Treatment groups				
		CG	E	H	LDP	HDP
	n:	5	5	4	6	5
Cholesterol (pg/dl)	B:	49	83.6	64.25	50.33	54
	A:	1421.6	1062.8	1251.2	1441.5	2282.67
	$\Delta = A-B^1$:	1372.6	979.2	1187	1391.17	2228.67
	Δ/Δ_{CG}:	1.00	0.71	0.86	1.01	1.62^3
Triglyceride (pg/dl)	B:	59.67	111	86.25	71	75.8
	A:	543.6	96.8	476.75	560.83	1035.33
	$\Delta = A-B^2$:	483.93	-14.2	390.5	489.83	959.53
	Δ/Δ_{CG}:	1.00	-0.03	0.81	1.01	1.98
Progesterone (ng/ml)	B:	—	—	—	0.28	0.2
	A:	—	—	—	3.03	6.48
	$\Delta = A-B^1$:	—	—	—	2.75	6.28

B--Before treatment; A--After treatment

1--All changes with $P<0.05$

2--All changes with $P<0.05$, except for the E group

3--$P<0.05$ when compared to the CG

Table 2. Morphometric Analysis of Plaque Load

	Treatment groups				
	CG	E	H	LDP	HDP
n:	6	5	4	6	6
Mean I/M:	0.81	0.02	0.29	0.42	0.47
STD:	0.43	0.04	0.39	0.21	0.25
$(I/M)/(I/M)_{CG}$:	1.00	0.02	0.29	0.42	0.47
P value*:	—	0.0001	0.0198	0.0001	0.0008

*when compared to the control group

330629

TABLE 3

ATHEROSCLEROTIC PLAQUE INHIBITION BY 17-OH PROGESTERONE

PLAQUE SIZE IN HYPERCHOLESTEROLEMIC RABBITS

VEHICLE (RABBIT#)

1	2	3	4	5	6
0.31	0.90	1.57	1.32	0.69	2.13

MEAN = 1.15

LOW DOSE (RABBIT#) (10 mg/kg)

7	8	9	10	11	12
0.66	0.30	0.84	0.85	0.14	0.52

MEAN = 0.55

HIGH DOSE (RABBIT#) (90 mg/kg)

13	14	15	16	17	18
1.37	0.11	0.33	0.23	0.18	0.02

MEAN = 0.37

5

EXAMPLE 2

Inhibition of Human Monocyte/Macrophage

Fcγ Receptor Mediated Phagocytosis

5

The agents to be tested (haloperidol, SCH-23390
and sulpiride) were incubated with human monocytes
isolated using lymphocyte separation medium.
Incubation was for 90 min at 37°C. Buffer (PBS)
10 treated cells served as a control. At the end of the
incubation period, monocytes were exposed to
IgG-sensitized cells in accordance with the phagocytic
assay of Indik et al, Blood 86:4389 (1995). The
results are as shown in Table 4.

15

Table 4

20

	%Phagocytic Cells[1]	PI[2]
Buffer (PBS) treated control	42%	105
Haloperidol (50 μM PBS/methanol)	0%	0
SCH-23390 (50 μM PBS/methanol)	16%	38
Sulpiride (500 μg/ml PBS/methanol)	59%	232

[1] % Phagocytic cells = Percent of monocytes/macrophages phagocytosing
IgG sensitized cells

30

[2] PI = Phagocytic index = The number of ingested IgG-sensitized cells per
100 monocytes.

No change in expression of the monocyte Fcγ receptor FcγRI, FcγRII or FcγRIIIA was noted using flow cytometry analysis.

5   *   *   *

All documents cited above are hereby incorporated in their entirety by reference.

One skilled in the art will appreciate from a reading of this disclosure that various changes in
10 form and detail can be made without departing from the true scope of the invention.